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## Supplementary information

# Dissimilatory sulfate reduction in the archaeon '*Candidatus* Vulcanisaeta moutnovskia' sheds light on the evolution of sulfur metabolism

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# Supplementary Material

## Dissimilatory sulphate reduction in archaeon *Candidatus* Vulcanisaeta moutnovskia sheds light on evolution of sulphur metabolism

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**Table S1.** Percentages of Archaea, Bacteria, and *Vulcanisaeta* representatives in the microbial community of Oreshek hot spring as assessed by qPCR

| Spring<br>pH<br>°C | "*Candidatus* V. moutnovskia" *dsrAB* genes in ng $DNA^{-}$ | *Archaea* 16S rRNA genes in ng $DNA^{-}$ | *Bacteria* 16S rRNA genes in ng $DNA^{-}$ | "*Candidatus* V. moutnovskia", portion in the community, %* | *Archaea,* % of total 16S rRNA genes | *Bacteria,* % of total 16S rRNA genes |
|---|---|---|---|---|---|---|
| Oreshek<br>3.5<br>91° | $3.0 \times 10^{5}$ +/- $5.1 \times 10^{4}$ | $7.22 \times 10^{5}$+/- $4.9 \times 10^{4}$ | $0.37 \times 10^{5}$ +/- $4.8 \times 10^{4}$ | 39.5 | 95 | 4.8 |

* Calculated as percent ratio of "*Candidatus* V. moutnovskia" *dsrAB* gene number in ng of tested DNA to the total 16S rRNA gene number in ng of tested DNA.

**Table S2**. Carbon source and electron acceptor utilization by binary culture 768-28 and the studied members of the Thermoproteaceae family

| **Microorganisms** | **Substrates** | **Electron acceptors** | | **Without acceptors** |
|---|---|---|---|---|
| | | **Sulphur** | **Sulphate** | |
| Binary culture 768-28 | Yeast extract | +* | +* | - |
| | Peptone | + | + | ND |
| | Beef extract | + | - | ND |
| | Fructose | + | - | ND |
| | Glucose | + | - | ND |
| | Sucrose | + | - | ND |
| | Maltose | + | - | ND |
| | Raffinose | + | - | ND |
| | Starch | + | - | ND |
| | Pyruvate | + | - | ND |
| *V. souniana* DSM 14430$^T$ | Yeast extract | +* | - | - |
| | Peptone | + | - | ND |
| | Maltose | + | - | ND |
| | Starch | + | - | ND |
| | Malate | + | - | ND |
| *V. distributa* DSM 14429$^T$ | Yeast extract | +* | - | - |
| | Peptone | + | - | ND |
| | Maltose | + | - | ND |
| | Starch | + | - | ND |
| | Mannose | + | - | ND |
| *T. tenax* DSM2078$^T$ | Yeast extract | +* | - | - |
| | Peptone | + | - | ND |
| | Glucose | + | - | ND |
| | Ethanol | + | - | ND |

| | | | | |
|---|---|---|---|---|
| | Fumarate | + | - | ND |
| *C. maquilingensis* DSM 13496$^T$ | Yeast extract | +* | - | - |
| | Peptone | + | - | ND |
| | Tryptone | + | - | ND |
| | Gelatine | + | - | ND |
| | Glycogen | + | - | ND |

Notes: ND stands for “not determined”. “-“ means lack of growth or about 2-fold increase in the cell number, which was sometimes observed, and was ascribed to the effect of a small amount of sulphur formed from oxidation of sulphide used as medium reductant, as the same effect was observed in medium without any electron acceptor added. “+*” means the authenticity of the grown cultures was checked by 16S rRNA gene sequencing.

**Table S3.** DSR-related proteins of "*Candidatus* V. moutnovskia" in the proteome analysis of soluble and membrane fractions. Normalized Exponentially Modified Protein Abundance Index (nemPAI) and protein content (mol%)*.

| Genbank locus tag (accession numbers) | Protein name | Annotation (NCBI) | nemPAI $SO_4$ | Protein content (mol%)* $SO_4$ | nemPAI $S_o$ | Protein content (mol%)* $S_o$ |
|---|---|---|---|---|---|---|
| VMUT_0502 (ADY00713) | DsrB | dissimilatory sulphite reductase subunit B | 0.023 | 2.3 | 0.001 | 0.1 |
| VMUT_0504 (ADY00715) | DsrC | dissimilatory sulphite reductase subunit C | 0.006 | 0.6 | 0.0001 | 0.01 |
| VMUT_0501 (ADY00712) | DsrA | dissimilatory sulphite reductase subunit A | 0.003 | 0.3 | 0.00001 | 0.001 |
| VMUT_0492 (ADY00703) | Sat | sulphate adenylyltransferase | 0.003 | 0.3 | 0 | 0 |
| VMUT_0500 (ADY00711) | DsrE | dissimilatory sulphite reductase subunit E | 0.002 | 0.2 | 0 | 0 |
| VMUT_0493 (ADY00704) | AprA | adenylylsulphate reductase, alpha subunit | 0.002 | 0.2 | 0.0002 | 0.02 |
| VMUT_0494 (ADY00705) | AprB | adenylylsulphate reductase, beta subunit | 0.001 | 0.1 | 0 | 0 |
| VMUT_0497 (ADY00708) | DsrK | dissimilatory sulphite reductase subunit K | 0.0005 | 0.05 | 0 | 0 |
| VMUT_2342 (ADY02534) | QmoA | heterodisulphide reductase, subunit A | 0.0002 | 0.02 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| VMUT_2341 (ADY02533) | QmoB | heterodisulphide reductase, subunit A | 0.0001 | 0.01 | 0 | 0 |
| VMUT_0635 (ADY00846) | "QmoAB-like" | 4Fe-4S ferredoxin, iron-sulphur binding domain protein | 0.0002 | 0.02 | 0 | 0 |
| VMUT_0498** (ADY00709) | DsrM | Nitrate reductase gamma subunit (NarI) | 0.0001 | 0.01 | - | - |
| VMUT_2339** (ADY02531) | QmoC | 4Fe-4S ferredoxin, iron-sulphur binding domain protein | 0.0001 | 0.01 | - | - |
| VMUT_1317** (ADY01522) | Pyrophosphatase | V-type H(+) translocating pyrophosphatase | 0.0005 | 0.05 | - | - |
| VMUT_1339** (ADY01544) | Sulphate transporter | ABC transporter ATP-binding protein | 0.00008 | 0.008 | - | - |

* - The mol% protein content was estimated from nemPAI[62] values.

** - Integral membrane proteins obtained with the denaturing extraction method are colored gray (see Extended Data Figure 7).

**Table S4.** Genomic dataset including genome accessions and taxonomic information.

(Excel file)

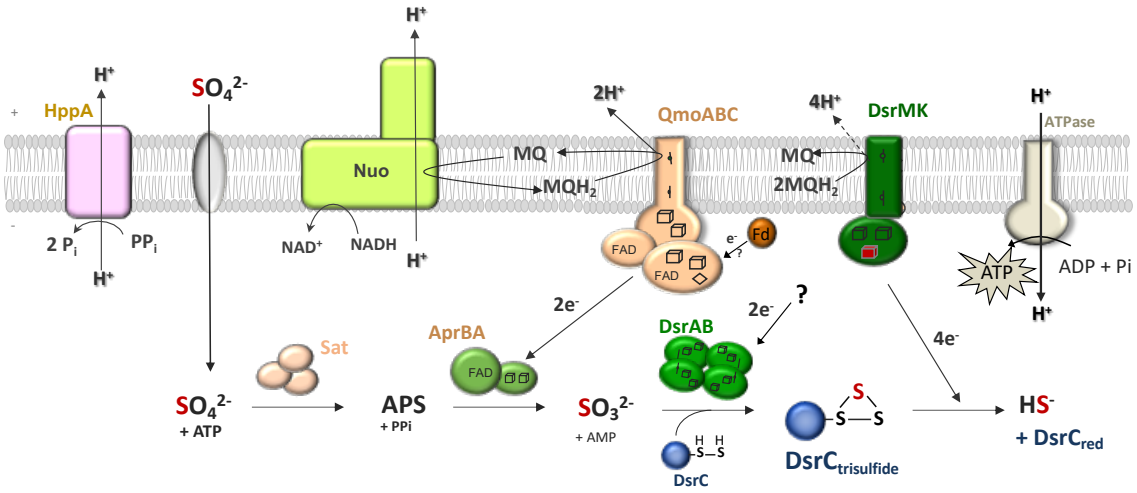


**Figure S1. Proposed mechanism of sulphate reduction in the hyperthermophilic archaeon "*Candidatus* V. moutnovskia".** Locus tags for the encoding genes are as follows: sulphate transporter: VMUT_0980; V-type $H^+$-translocating pyrophosphatase: VMUT_1317; Sulphate adenylyltransferase (Sat): VMUT_0492 (ADY00703); Adenylylsulphate reductase (AprAB): VMUT_0493 (ADY00704), VMUT_0494 (ADY00705); Sulphite reductase: DsrA - VMUT_0501 (ADY00712), DsrB - VMUT_0502 (ADY00713), DsrC - VMUT_0504 (ADY00715), DsrK - VMUT_0497 (ADY00708), DsrM - VMUT_0498 (ADY00709); Quinone-interacting membrane-bound oxidoreductase complex (QmoABC): VMUT_2342 (ADY02534), VMUT_2341 (ADY02533), VMUT_2339 (ADY02531); V-type $H^+$-transporting $A_1A_0$-ATP synthase subunits AtpABCDEFIK: VMUT_1268 (ADY01473), VMUT_0931 (ADY01141), VMUT_0511 (ADY00722), VMUT_0599 (ADY00810), VMUT_1269 (ADY01474), VMUT_1278 (ADY01483), VMUT_1733 (ADY01935), VMUT_0983 (ADY01193); NADH:quinone oxidoreductase subunits NuoABCDEFGHIJKLMN: VMUT_2103 (ADY02300), VMUT_2104 (ADY02301), VMUT_2105 (ADY02302), VMUT_0816 (ADY01026), VMUT_0815 (ADY01025), VMUT_0941 (ADY01151), VMUT_1959 (ADY02160), VMUT_0559 (ADY00770), VMUT_0558 (ADY00769), VMUT_1814 (ADY02015), VMUT_1815 (ADY02016), VMUT_0557 (ADY00768).